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HEMATOLOGIC CHARACTERIZATION OF NATURALLY OCCURRING MALARIA (PL--ETC(U)
APR 82 J C DONOVAN, W S STOKES, R D MONTREY

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Hematologic Characterization of Naturally Occurring Malaria (*Plasmodium inui*) in the Cynomolgus Monkey (*Macaca fascicularis*)[1-6]

Short Title: Effects of Malaria in the Cynomolgus Monkey

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[2]The views of the authors do not purport to reflect the positions of the Department of the Army or Department of Defense.

[3]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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[6]The authors would like to thank R. Blackman, D. Miller, T. Tyler, J. Watson, and R. Wright for technical assistance.

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Summary Forty-three percent of 47 recently imported cynomolgus monkeys (Macaca fascicularis) were found to have malarial infections. The agent identified was Plasmodium inui. All infections were subclinical in nature. Parasitemias ranged from 10-900 parasites/mm^3 of whole blood. Pre- and posttreatment hematologic values were evaluated following treatment with chloroquine. Treatment was effective in clearing parasitemias from 13 of 14 infected monkeys. Pretreatment values of hematocrit, hemoglobin and mean corpuscular volume were significantly different in infected animals compared to noninfected animals. While posttreatment hemoglobin and hematocrit values returned to noninfected control levels, mean corpuscular volume values of infected animals remained significantly lower in the posttreatment period.
Key Words: Plasmodium - Malaria - Macaca fascicularis - Chloroquine

Malaria is a naturally occurring infectious disease of mammals, birds and reptiles which manifests itself as a syndrome with various degrees of anemia, fever, malaise and splenomegaly. Infection is caused by blood-borne protozoan parasites of the genus Plasmodium. The parasite is transmitted by the bite of infected mosquitoes. Following a stage of exoerythrocytic schizogony in the liver of the mammalian host, merozoites are released into the bloodstream and subsequently parasitize circulating erythrocytes. In the erythrocyte, an asexual reproductive cycle is established, with hemoglobin being utilized by the developing parasites. Infected red blood cells are ruptured by the developing intracellular parasites or removed from circulation by the reticuloendothelial system.

Various species of Plasmodium, including P. inui, P. cynomolgi, P. knowlesi, P. coatneyi and P fieldi are known to infect the cynomolgus monkey and mixed infections are common (1,2). The incidence of infection is dependent on vector availability and host-parasite antigenic relationships (1,3,4). While an abundance of information is available with reference to nonhuman primate malarias, there is a lack of published data evaluating the effects of naturally occurring malaria infections on the hematologic values of the cynomolgus host.

The impetus for this investigation was provided by: (a) the importance to the research community of critically defining intercurrent disease processes in experimental animals; (b) the growing role of the cynomolgus monkey in biomedical research and; (c) the widespread use of hematologic values as indicators of physiologic response in a variety of research endeavors. The purpose of this investigation was: (a) to determine the incidence, parasite counts and etiologic agent of malaria infections in cynomolgus monkeys recently imported to the United States;

(b) to characterize the effects of the infections on the hematologic values of this primate species; and (c) to evaluate treatment with chloroquine.

Materials and Methods

Animals -- Forty-eight, adult, male Macaca fascicularis were obtained from a commercial importer[8] following a minimum 45-day conditioning period. The countries of origin were the Philippines and Indonesia. One monkey died within hours of arrival at our quarantine facility of an undiagnosed illness. The remaining 47 animals were found to be clinically normal by physical examination and daily observation for 2 weeks. Animals were housed indoors in aluminum squeeze cages, fed a commercial monkey chow[9] twice a day with twice weekly fruit supplementation and given water ad libitum via an automatic watering system. Room environment was maintained at 24 $\pm$ 1 C and 40 $\pm$ 10% relative humidity with a 12-hour light-dark cycle.

Experimental design -- The investigation was scheduled for a 6-week period beginning 2 weeks after the animals' arrival at our quarantine facility. During the first week of study blood samples were collected on 3 consecutive days to determine incidence, parasite identity and parasite counts. With this initial information, 14 infected and 14 noninfected animals were randomly selected for study. Blood samples were taken for hematologic determinations and parasite counts at 1 week intervals throughout the study period. Hematologic determinations included total erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume and total and differential leukocyte counts. Reticulocyte counts were not performed, as previous experience at this

[8] Worldwide Primates, Inc., Miami, FL

[9] Lab Chows, High Protein Monkey Chow, Ralston Purina Company, St. Louis, MO

institute had failed to demonstrate a reticulocyte response at the levels of parasitemias encountered. All infected animals received 5 mg/kg chloroquine base[10] intramuscularly once a day for 7 days between the samplings in the third and fourth week. Nine of the other noninfected animals were also treated with this regime.

Technical procedures -- All blood collection was performed using ketamine hydrochloride,[11] intramuscularly, 5 mg/kg, for restraint. Blood samples for parasite counts and morphologic identification were obtained by inserting a 25-gauge needle into the saphenous vein and collecting from the hub of the needle using heparinized microhematocrit capillary tubes. Thick and thin Giemsa-stained blood smears were used to identify the species of malaria and establish parasite counts. Determination of parasite identity was based on morphologic characterization on thin smears. Blood samples for complete blood counts were collected with a 20-gauge needle from the femoral vein into 2-ml vacuum tubes[12] containing EDTA as an anticoagulant. Hematologic determinations were performed with the use of an automated blood cell counter and hemoglobinometer.[13]

Statistical analysis -- Statistical analysis was performed using the analysis of variance (5). Effects of treatment on infected monkeys were measured and analyzed. The effects of infection between malaria positive monkeys and noninfected monkeys were also compared. Treatment effects were analyzed by comparing the mean of three pretreatment values to each of three posttreatment values using a completely randomized design. Effects of infection were analyzed separately for the pre- and posttreatment periods utilizing a completely randomized design with two factorials. Differences between treated and untreated noninfected monkeys were also measured. Significance was defined at $p \leq 0.05$.

[10]Aralen[R], Winthrop Laboratories, Division of Sterling Drug, Inc., New York, NY

[11]Vetalar[R], Parke-Davis, Detroit, MI

[12]Vacutainer[R], Becton, Dickinson, and Company, Rutherford, NJ

[13]Coulter Electronics, Inc., Hialeah, FL

Results

The etiologic agent of patent infections was in all cases P. inui. Forty-three percent of the 46 monkeys were found to be infected. Single-day determinations resulted in incidence rates of 31, 28 or 21%, while accumulated data from the 3 consecutive days gave the reported rate of 43%. All infections were subclinical in nature with parasitemias ranging from 10-900 parasites/mm^3 of whole blood. Treatment was successful in clearing parasitemias in 13 of 14 monkeys, with no observable adverse side-effects. The one nonresponder and 6 infected monkeys not included in the study were allowed to remain infected for further clinical studies.

Hematocrit, hemoglobin, mean corpuscular volume, and erythrocyte values were lower in infected than in noninfected monkeys before treatment (Figure 1). This difference was significant at the $p < 0.05$ level in all except the erythrocyte count (Table 1). The hematocrit, hemoglobin and erythrocyte counts of infected monkeys showed a significant increase following treatment, to where there were no longer significant differences between them and noninfected monkeys. The mean corpuscular volume of infected animals remained at the same level for the 3-week posttreatment period observed, which was still significantly lower than the noninfected monkeys. No significant differences were found between treated and untreated noninfected monkeys throughout the study period; the noninfected monkeys were thus considered a homogeneous group for subsequent analyses. Although total and differential leukocyte counts were also measured, within group variability was great and no significant differences were noted between any of the groups before or after treatment.

Discussion

This investigation was conducted in order to characterize in part the effects of naturally occurring malaria in cynomolgus monkeys by observing alterations of hematologic values in infected animals. The data presented in Figure 1 show that cynomolgus monkeys may have significant hematologic alterations associated with low grade malarial infections. Table 1 presents the significance of the findings. Although these clinically inapparent infections did not uniformly cause fluctuations of hematologic values outside the published normal range (6), the alterations are significantly different from noninfected control values and clearly compromised the animals' utility in research.

The changes in erythrocyte count, hematocrit and hemoglobin are relatively straightforward in malaria infections and are associated with the destruction of erythrocytes by developing intracellular parasites or removal of altered red blood cells by the reticuloendothelial system (6). An explanation for the reduction of mean corpuscular volume of infected animals is more difficult. Microcytic anemias are generally caused by the effects of chronic infectious disease or iron deficiency. The chronic malaria infection may serve as an explanation; however, iron deficiency was not ruled out. Additional studies are necessary to determine the precise nature and cause of the erythrocytic microcytosis seen here.

Further studies are necessary to evaluate other potential physiologic and pathologic alterations caused by malarial infection which may have an impact on biomedical research. In particular, attention has been given to immunologic alterations caused by malaria in experimental and natural infections of man and nonhuman primates (7,8).

A practical lesson regarding diagnosis of malaria was learned during this investigation. The parasitemia observed in many cases was extremely low with this subclinical manifestation in the cynomolgus monkey, with perhaps daily fluctuation caused by the periodicity of the organism. Because of these reasons, an accurate accounting of positively infected animals can only be obtained by examining blood on at least 3 consecutive days. Our results revealed a higher incidence of infection by this method than any single daily determination would have shown.

When treating nonhuman primate malaria, drug selection must be predicated on accurate species identification of the organism in order to insure efficacy. This is particularly true of the cynomolgus monkey in which the various malarias may or may not have a persistant tissue phase. The potential for mixed infections further complicate drug selection. In this study only pure infections of P. inui were observed. Chloroquine, a drug effective in treating non-relapsing malarias, was successful in clearing parasitemias. P. inui is a quartan-type malaria without a persistent tissue phase and thus was expected to be susceptible to chloroquine treatment.

Based on these results, we recommend that all cynomolgus monkeys from malaria endemic areas be screened for malaria before use in biomedical research. Species identification of the malaria parasite and appropriate treatment should be instituted and follow-up blood smears examined to insure efficacy of treatment. Investigators contemplating the use of wild-caught cynomolgus monkeys should be made aware of the occurrence and consequences of this disease. If treatment is instituted prior to use, investigators should also be informed of the antimalarial drug utilized and its potential adverse side-effects.

References

1. Coatney GR, Collins WE, Warren MW, et al. The Primate Malarias. US Government Printing Office, Washington, DC, 1971.

2. Ruch TC. Diseases of Laboratory Primates. W. B. Saunders, Philadelphia, 1959.

3. Coatney RC. The simian malarias: zoonoses, anthroponoses, or both? Am J Trop Med Hyg 20:795-803, 1971.

4. Eyles DE. The species of simian malaria: taxonomy, morphology, life cycle and geographical distribution of the monkey species. J Parasitol 49:866-887, 1963.

5. Daniel WW. Biostatistics: A Foundation for Analysis in the Health Sciences. John Wiley and Sons, New York, 1978.

6. Benirschke K, Garner FM, Jones TC. Pathology of Laboratory Animals, p 1001-1009, 1610-1618. Springer-Verlag, New York, 1978.

7. Greenwood, BM. Immunosuppression in Malaria and Trypanosomiasis in Parasites in the Immunized Host: Mechanisms of Survival. Ciba Foundation Symposium 25:137-146. Associated Scientific Publishers, Amsterdam, 1974.

8. Voller A. Immunopathology of malaria. Bull WHO 50:177-186, 1974.

Table 1

Significance ($p <$) of Observed Values

Parameter	Infected monkeys, pretreatment mean vs. posttreatment study week			Infected vs noninfected	
	4	5	6	Pre-treatment	Post-treatment
Hematocrit (%)	0.05	0.05	0.01	0.01	NS
Hemoglobin (g/dl)	0.05	0.01	0.001	0.01	NS
Erythrocytes ($no./mm^3 \times 10^6$)	NS	0.05	0.01	NS	NS
Mean corpuscular volume (μm^3)	NS	NS	NS	0.05	0.05

FIGURE LEGEND

Figure 1

Hematologic values, pre and posttreatment of 14 noninfected (O— —O) and 14 infected (●— —●) cynomolgus monkeys.